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Morpho-cultural variability of different *Rhizoctonia solani* isolates associated with banded leaf and sheath blight disease of kodo millet.

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ABSTRACT

In the current study the *Rhizoctonia solani* isolates were obtained from different millet-producing regions in India where BLSB incidence was more predominant. They were cultured on PDA in order to observe the morpho- cultural characters Radial growth was measured at 24 h interval time from 2 to 5 DAI and was found that the highest growth rate was observed in SGK-1 (0.91 mm h^{-1}) and the lowest in KRK-1 (0.64 mm h^{-1}) and two distinct mycelial patterns were observed *i.e*., profusely growing cottony fluffy and profusely growing cottony; with colony colour varying from light yellowish brown to very pale brown. The range and hyphal width measured between 5.65 – 9.66 mm whereas, size of sclerotia ranged between 0.92 mm to 1.69 mm. Initiation of sclerotia was observed at 3 to 4 DAI and a prominent variation was seen in sclerotial pattern of arrangement. Two different types of sclerotial distribution was recorded *i.e*., surface and surface and touch the lid where both micro and macro type of sclerotia were observed. Variations in sclerotia colour (reddish brown to dark brown), sclerotia number (54.67 to 191.67) and weight of sclerotia (28.3 mg to 85.2 mg) were also noticed on PDA

Keywords: *BLSB, Morpho- cultural, Mycelia, Rhizoctonia solani and Sclerotia*

Millets are small-seeded annual crops grown as cereal grains. Of them kodo millet, an ancient grain, which is originated in Africa and was domesticated in India thousands of years ago is known for its drought resistance (Tonapi *et al*., 2022). Kodo millet belongs to the genus *Paspalum*, family Poaceae and the sub-family Panicoideae (Ravikesavan *et al*., 2023) and includes 400 species that extend across the warmer regions of the world. Cultivated plants are mostly grown as annuals, but many cultivars grow at the lower nodes and under favourable conditions they continue to produce culms after the older shoots have flowered and matured their inflorescences and occurs in moist or shady places across the tropics and subtropics (de Wet *et al*.,1983). In India, its distribution is primarily concentrated in Deccan region and its cultivation is confined to Gujarat, Karnataka, Madya Pradesh, Maharashtra and parts of Tamil Nadu and also spread to the foothills of Himalayas. Madhya Pradesh and Tamil Nadu have the maximum share in production and promotion of kodo millet (Jalajakshi *et al*., 2022).

Several diseases (rust, ergot, blast, brown spot, foot rot) were reported in kodo millet among them banded leaf and sheath blight caused by *Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*) is the major devastating disease. The disease results in substantial yield losses of about 49.3-57.7% (Patro *et al*., 2021). Characteristic symptoms of the disease are formation of large irregular lesions that have straw coloured center with wide reddish-brown margin (Kahar, 2017). Under favourable conditions, lesions enlarge rapidly, coalesce to cover large portions of the sheath and leaf blade. At this stage, the disease symptoms are characterized by a series of copper brown colour bands across the leaves and sheath giving a characteristic banded appearance (Jain. 2024). The presence of several large spots on the leaf sheath gradually causes the death of the whole leaf. The pathogen persists in the soil through infected plant debris left in the field following crop harvest and also by forming the dormant survival structure sclerotia during unfavourable conditions (Bashyal *et al*., 2021). Keeping the above information in view, the present study is thus aimed to collect the *R. solani* isolates

associated with banded leaf and sheath blight of kodo millet and characterize them both morphologically and culturally.

MATERIAL AND METHODS

Samples of *R. solani* affected by banded leaf and sheath blight in kodo millet were collected from different states of India like Andhra Pradesh, Orissa, Karnataka, Tamil Nadu, Chhattisgarh, Uttarakhand and the respective isolates were named after them. The disease plant samples along with some healthier portions were cut into small bits and sterilized in 1% sodium hypochlorite (NaOCl) solution, which was then rinsed thrice with sterilized distilled water and dried on sterilized filter paper. Three or four small surface sterilized infected leaf bits were inoculated in each petriplates at equal distance containing Potato Dextrose Agar (PDA) and incubated at 27±1°C in three replications. Later, the obtained cultures were subcultured by hyphal tip method. Subsequently, pure cultures were maintained for further examination. A 5 mm mycelial disc from the three-day-old culture was placed in the center of PDA in triplicate and incubated at 27±1^{0}c for 4 days.

The morphological and cultural traits were examined for each isolate, with the mean value of every characteristic recorded. Cultural traits like colony diameter (measured every 24 hours), and the growth rate, as well as hyphal width, colony texture and colour, were evaluated appropriately for each isolate to assess their cultural features. Morphological traits such as sclerotia colour were classified into different categories: reddish brown, brown, and dark brown. The distribution was recorded as surface and touch the lid, while the pattern of arrangement were categorized as central, sub-central, both central and sub-central, peripheral ring, and scattered. Sclerotial weight and size measurements were taken from 25 observations for each isolate, and sclerotial texture was classified into rough and smooth types, along with a count of the number of sclerotia for each isolate were recorded. Hyphal Width was calculated by taking four-day old fungal hyphae from four corners of culture plate were used to measure hyphal width of the isolates. They were stained with 0.1 % lactophenol cotton blue and a total of 25 observations was recorded from each isolate (Lal and Kandhari, 2009). The following formula was employed for comparison. $\mu = X \pm t25(0.05) \times S.E$ Where, μ = hyphal width X = mean of 25 observations t25(0.05) = 1.96 S. E = Standard error of the mean.

Table 1. Variation in mycelial growth among different isolates of *Rhizoctonia solani* on PD

S. No	Isolate	Colony diameter (cm)				Growth rate (mm h^{-1})				Average growth rate (mm h^{-1})
		2 DAI	3 DAI	4 DAI	5 DAI	2 DAI	3 DAI	4 DAI	5 DAI	
1	VZK-1	3.87	5.47	8.5	9	0.81	0.76	0.89	0.75	0.8
2	VZK-2	2.66	6.1	7.8	9	0.56	0.85	0.81	0.75	0.74
3	NDK-1	5	5.67	7.83	8.85	1.04	0.79	0.82	0.71	0.84
4	NDK-2	3.5	5.7	8.47	9	0.73	0.79	0.88	0.75	0.78
5	JDK-1	4.36	6.77	8.87	9	0.91	0.94	0.92	0.75	0.88
6	JDK-2	3.37	6.13	7.17	9	0.7	0.85	0.75	0.75	0.76
7	SGK-1	4.4	7.53	9	9	0.92	1.05	0.94	0.75	0.91
8	ARK-1	3.3	6.67	8.53	9	0.69	0.93	0.89	0.75	0.81
9	RNK-1	4.13	6.9	8.9	9	0.86	0.96	0.93	0.75	0.87
10	KRK-1	2.63	4.6	6.63	8.82	0.55	0.64	0.69	0.7	0.64
11	MDK-1	4.2	5.93	7.43	9	0.88	0.82	0.77	0.75	0.8
12	HGK-1	3.43	5.87	7.37	9	0.72	0.82	0.77	0.75	0.76
13	AMK-1	3.6	6.47	8.93	9	0.75	0.9	0.93	0.75	0.83
14	BAK-1	3.03	6.2	8.57	9	0.63	0.86	0.89	0.75	0.78
15	BAK-2	3.73	6.8	8.87	9	0.78	0.94	0.92	0.75	0.84
	CD ($P \leq 0.05$)	0.52	0.78	0.51	0.17					
	CV (%)	8.4	7.57	3.71	1.11					

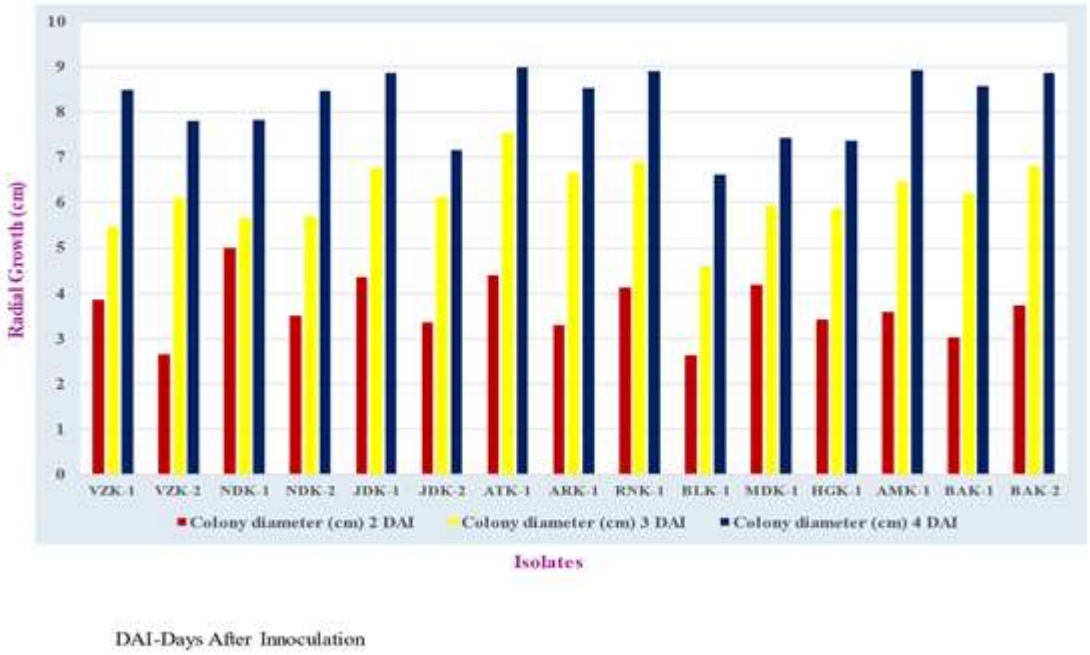

Fig 1. Radial growth of *Rhizoctonia solani* isolates at different days after inoculation on PDA

Table 2 Variation in morpho- cultural characterization of *R. solani* isolate on PDA

S. No	Isolate	Colony texture	Colony Colour*	Colour of sclerotia
1	VZK-1	Profusely growing cottony aerial mycelium	Very Pale Brown	Reddish Brown
2	VZK-2	Profusely growing cottony aerial mycelium	Very Pale Brown	Dark Brown
3	NDK-1	Profusely growing cottony fluffy aerial mycelium	Very Pale Brown	Dark Brown
4	NDK-2	Profusely growing cottony aerial mycelium	Light Yellowish Brown	Dark Brown
5	JDK-1	Profusely growing cottony aerial mycelium	Light Yellowish Brown	Reddish Brown
6	JDK-2	Profusely growing cottony fluffy aerial mycelium	Yellowish Brown	Dark Brown
7	SGK-1	Profusely growing cottony aerial mycelium	Very Pale Brown	Dark Brown
8	ARK-1	Profusely growing cottony fluffy aerial mycelium	Yellowish Brown	Light Brown
9	RNK-1	Profusely growing cottony aerial mycelium	Very Pale Brown	Dark Brown
10	KRK-1	Profusely growing cottony aerial mycelium	Very Pale Brown	Light Brown
11	MDK-1	Profusely growing cottony fluffy aerial mycelium	Yellowish Brown	Dark Brown
12	HGK-1	Profusely growing cottony aerial mycelium	Very Pale Brown	Light Brown
13	AMK-1	Profusely growing cottony fluffy aerial mycelium	Very Pale Brown	Reddish Brown
14	BAK-1	Profusely growing cottony fluffy aerial mycelium	Yellowish Brown	Reddish Brown
15	BAK-2	Profusely growing cottony fluffy aerial mycelium	Yellowish Brown	Reddish Brown

RESULTS AND DISCUSSION

Significant differences were observed among *R. solani* isolates of kodo millet in terms of colony diameter and growth rate on PDA medium. Mycelial growth measurements were taken at 24-hour intervals starting from 2 days after inoculation (DAI). At 2 DAI, maximum growth was recorded in isolate NDK-1 (5.00 cm), followed by SGK-1 (4.40 cm) and JDK-1 (4.36 cm), while KRK-1 (2.63 cm) and VZK-2 (2.66 cm) showed the least growth. By 3 DAI, SGK-1 (7.53 cm) led in growth which is on par with RNK-1, BAK and JDK-1, whereas, KRK-1 (4.60 cm) remained as slowest. At 4 DAI, SGK-1 achieved full plate growth (9.00 cm). However, by 5 DAI all the isolates have attained full growth (9.00 cm) except NDK-1 (8.55 cm) AND KRK-1(8.82 cm). Further, SGK-1 showed the highest average growth rate (0.91 mm/h), followed by JDK-1 (0.88 mm/h), while KRK-1 showed the lowest (0.64 mm/h) (Table-1; Fig-1; Fig 2). These findings align with earlier studies (Gopireddy *et al*., 2017; Meena *et al*., 2003; Kipsumbai *et al*., 2022; Mishra *et al*., 2014; Thind

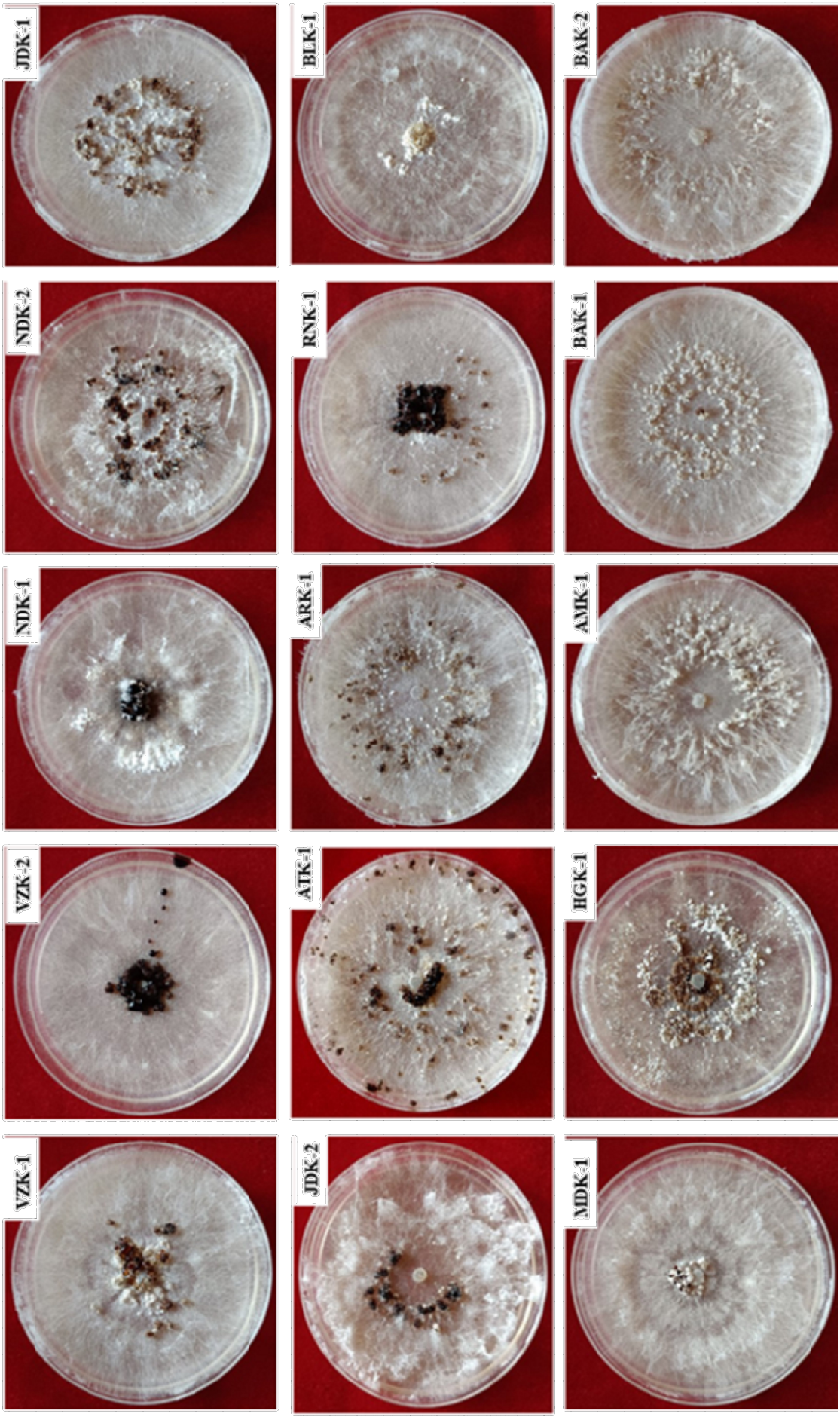

Plate 1. Variability of *Rhizoctonia solani* isolates on PDA medium

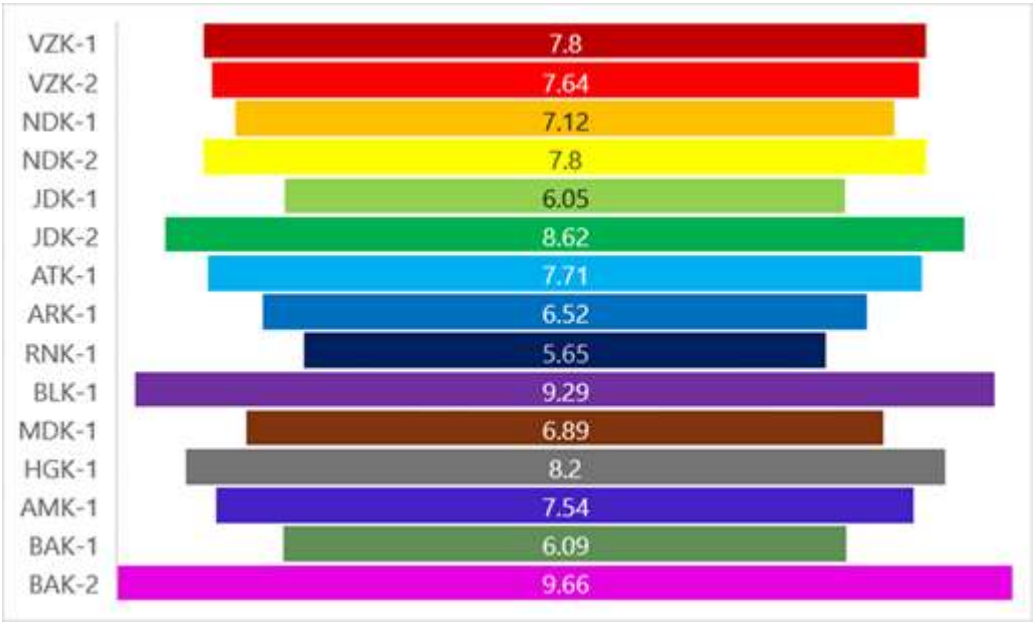

Fig 2. Variations in hyphal width of *Rhizoctonia solani* isolates on PDA

& Aggarwal, 2008; Sharma. 2005) which reported significant cultural variation and linked faster growth with higher virulence. Two distinct mycelial growth types *i.e*., profusely growing cottony fluffy (NDK-1, JDK-2, ARK-1, MDK-1, AMK-1, BAK-1 and BAK-2) and profusely growing cottony (VZK-1, VZK-2, NDK-2, JDK-1, SGK-1, RNK-1, KRK-1 and HGK-1) (Table-2) were observed with aerial mycelium. Initially, colony colour was white, later showed variations from 10 DAI. Isolates NDK-2 and JDK-1 developed light yellowish brown colonies, while JDK-2, ARK-1, MDK-1, BAK-1 and BAK-2 showed yellowish brown and the rest exhibited very pale brown colour as per Munsell's soil colour chart. These variations were in accordance with earlier findings of Budiarti *et al*. (2020), Singh *et al*. (2014), Kipsumbai *et al*. (2022), suggesting that colony colour differences may result from pigment or secondary metabolite production by the pathogen.

The hyphal width, among the isolates ranged from5.66 µm to 9.66 µm. The maximum hyphal width of 9.66 µm was recorded in BAK-2 which was on par with KRK-1 (9.29 µm) where minimum hyphal width was observed in RNK-1 (5.65 µm) which was on par with JDK-1 (6.05 µm), BAK-1(6.09 µm) and ARK-1 (6.52 µm) (Table -3; Fig-2; Plate-2). The similar results were reported by Nabi *et al*. (2024) in rice where the hyphal width of *R. solani* among 50 isolates ranged from 4.96-9.55µm.

Dimension of the sclerotia varied from 0.92 mm and 1.69 mm (Fig-3), Basu *et al*. (2004), described the diameter of sclerotia ranged from 0.23 to 1.91 mm in Rice. Sclerotial formation was observed from 3 to 5 DAI and the texture of sclerotia was classified as rough and smooth. Among the 15 isolates studied, majority of the isolates (VZK-1, NDK-2, JDK-1, JDK-2, SGK-1, ARK-1, RNK-1, KRK-1, HGK-1, BAK-1) formed rough textured sclerotia. Whereas, the remaining five isolates (VZK-2, NDK-1, MDK-1, AMK-1, BAK-2) formed smooth textured sclerotial bodies. Among 15 isolates of *R. solani* sclerotial number was varied from 191.67 to 54.67. The maximum sclerotial bodies (191.67) was formed in SGK-1 followed by HGK1(185.33), BAK-1 (128.33), NDK-2 (114.33) and ARK-1 (113.67) whereas, the least sclerotial number in VZK-2 (54.67). A mean weight of 25 sclerotial bodies were considered from each isolate. Sclerotial weight was ranged from 28.3 mg - 85.2 mg (Table 4.6) The maximum sclerotial weight 85.2 mg was found in Karnataka isolate MDK-1 followed by Uttarakhand isolate AMK-1 (60.1 mg) and Orissa isolate BAK-1 (58.2 mg). Whereas, the minimum sclerotial weight (28.3 mg) was recorded in (KRK-1). Sangeeta *et al*. (2021) retrieved eleven isolates of the sheath blight pathogen from the major rice cultivation regions in India and noted that the weight of the sclerotia varied between 0.116 and 0.373 g. (Table 4, Fig- 4). Type of sclerotia varied as macro and micro sclerotia (Plate-3)

Plate 2. Variation in hyphal width among different isolates of *Rhizoctonia solani* (400X)

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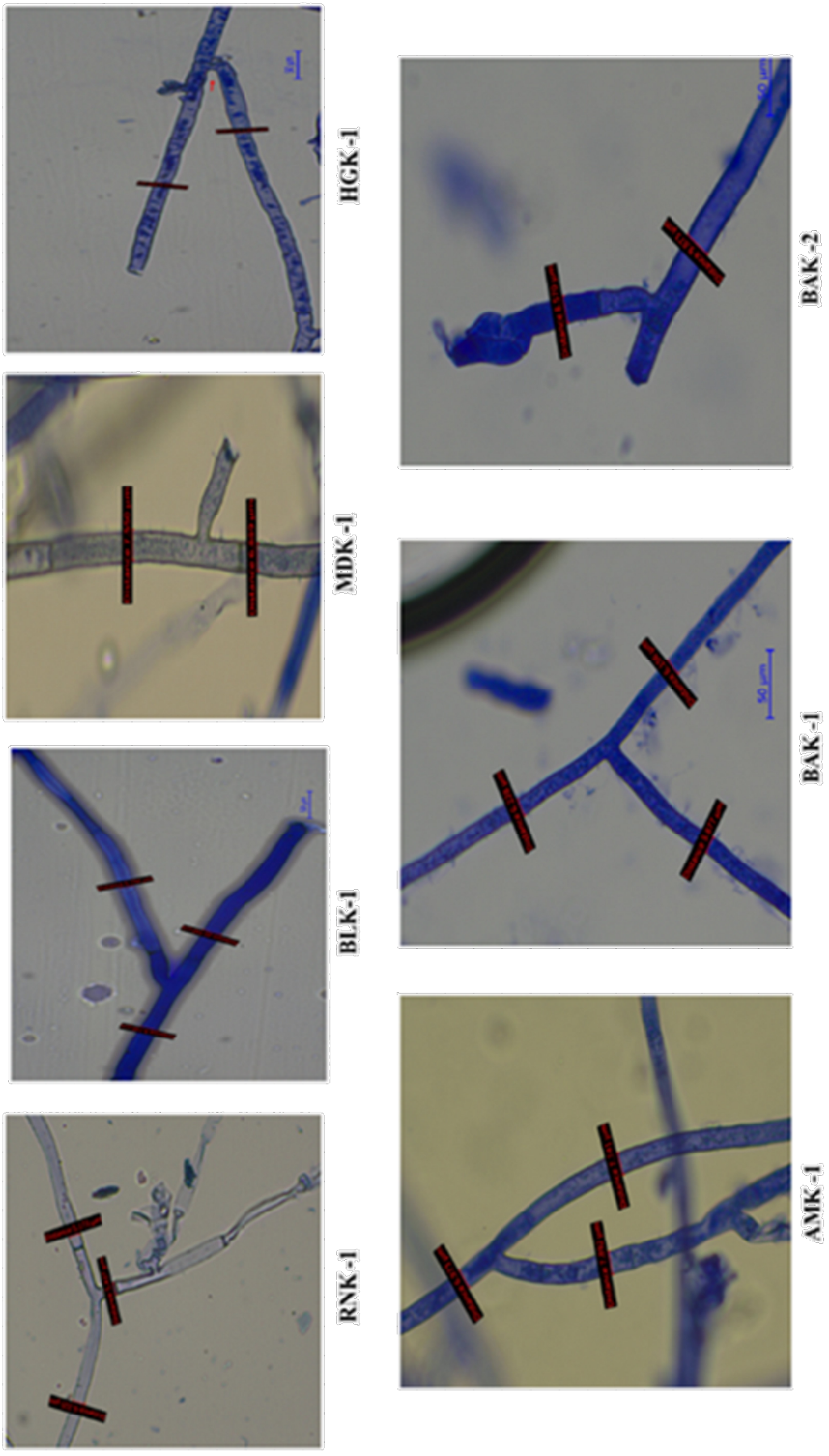

Plate 2. Variation in hyphal width among different isolates of *Rhizoctonia solani* (400X)

Table 3. Variation in Characteristics of sclerotia among different isolates of *Rhizoctonia solani* on PDA

S. No	Name of the isolate	Days for sclerotia initiation	Sclerotia distribution	Pattern of arrangement	Sclerotia texture	Type of sclerotia	Sclerotia categorization	Sclerotia number	Sclerotia weight(mg)
1	VZK-1	4 DAI	Surface	Irregular	Rough	Macro	Excellent	69	52
2	VZK-2	4 DAI	Surface	Centre	Smooth	Macro	Very Good	55	56.8
3	NDK-1	3 DAI	Surface	Sub-Central ring	Smooth	Macro	Excellent	87	37.8
4	NDK-2	4 DAI	Surface and touch the lid	Scattered	Rough	Macro	Excellent	114	43.9
5	JDK-1	4 DAI	Surface	Sub-Central ring	Rough	Macro	Excellent	99	41.8
6	JDK-2	4 DAI	Surface and touch the lid	Irregular	Rough	Micro	Very Good	61	33.4
7	SGK-1	4 DAI	Surface and touch the lid	Scattered	Rough	Micro	Excellent	192	32.5
8	ARK-1	4 DAI	Surface and touch the lid	Irregular	Rough	Micro	Very Good	114	56.8
9	RNK-1	4 DAI	Surface	Centre and scattered	Rough	Micro	Excellent	70	36.9
10	KRK-1	4 DAI	surface	Centre	Rough	Micro	Excellent	100	28.3
11	MDK-1	4 DAI	Surface	Centre	Smooth	Macro	Excellent	100	85.2
12	HGK-1	3 DAI	Surface	Sub-Central ring	Rough	Micro	Excellent	185	44.7
13	AMK-1	4 DAI	Surface and touch the lid	Peripheral ring	Smooth	Macro	Excellent	97	60.1
14	BAK-1	4 DAI	Surface	Sub-Central ring	Rough	Macro	Excellent	128	58.2
15	BAK-2	4 DAI	Surface and touch the lid	Peripheral ring	Smooth	Macro	Excellent	91	46.6

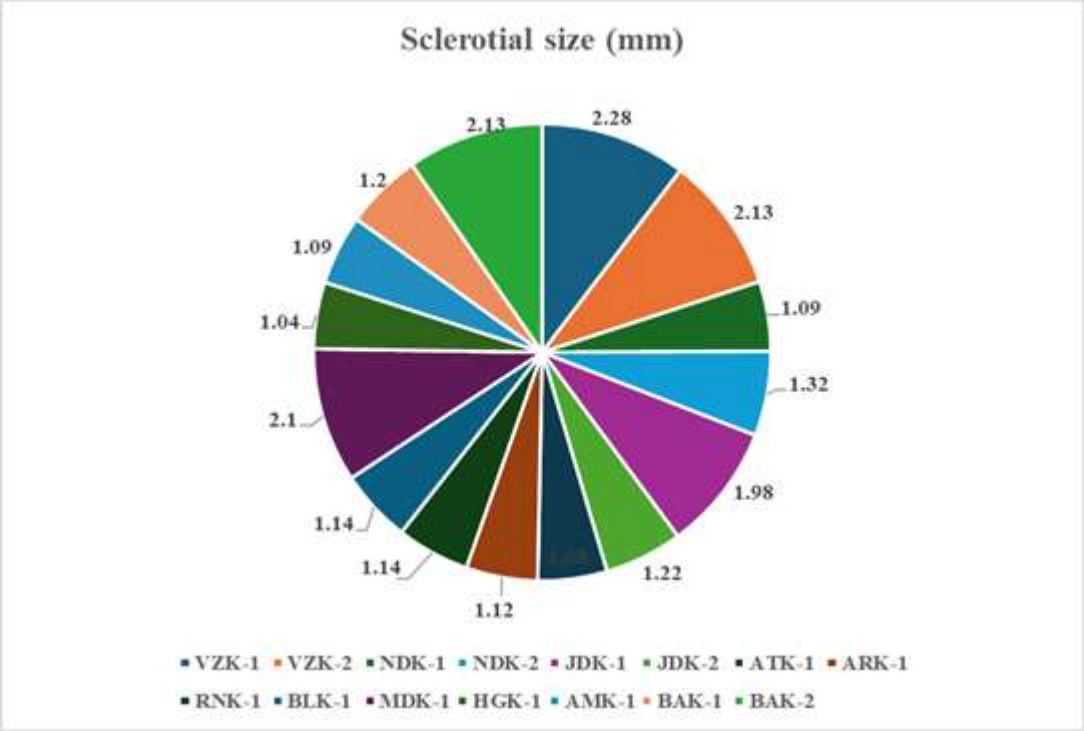

Fig 3 Variations in sclerotial size of *Rhizoctonia solani* isolates on PDA

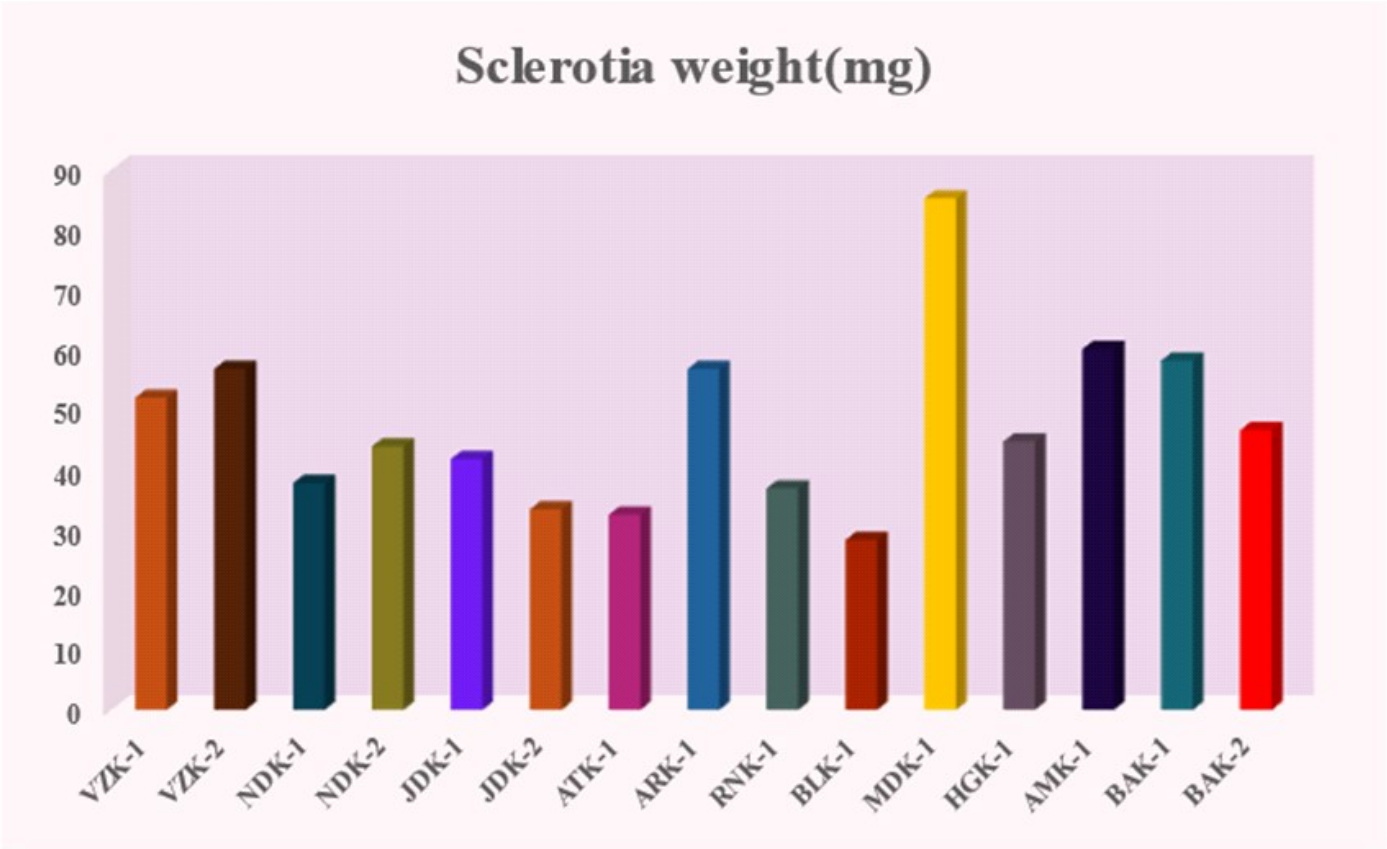

Fig 4 - Variations in sclerotial weight of *Rhizoctonia solani* isolates on PDA

CONCLUSION

In this present investigation 15 isolates have generated set of information where we could observe great diversity among the same plant which were collected from different locations of India in morphological and cultural characteristics. Differences in morphological and cultural traits were observed among the fifteen isolates. The highest average growth rate was found in isolate SGK-1 (0.91mm h^{-1}) followed by Chhattisgarh isolate JDK-1 (0.88 mm h^{-1}) whereas, lowest average growth rate was found in isolate KRK-1 (0.64 mm h^{-1}).

At 7 DAI, *R. solani* isolates showed variation in colony texture and colour on PDA. Two distinct mycelial growth patterns: cottony fluffy (NDK-1, JDK-2, ARK-1, MDK-1, AMK-1, BAK-1 and BAK-2) and cottony (VZK-1, VZK-2, NDK-2, JDK-1, SGK-1, RNK-1, KRK-1, HGK-1). All the isolates showed aerial mycelium. Initially, colony colour was white which showed variations from 10 DAI. Based on Munsell's soil colour chart, colony colour and individual isolates differentiated as Light yellowish brown (NDK-2 and JDK-1), yellowish brown (JDK-2, ARK-1, MDK-1, BAK-1 and BAK-2) and very pale brown (VZK-1, VZK-2, NDK-1, SGK-1, RNK-1, KRK-1, HGK-1, AMK-1).

The hyphal width among the isolates ranged from 5.65 µm to 9.66 µm. The maximum width (9.66 µm) was observed in BAK-2, which was on par with KRK-1 (9.29 µm) and the minimum width was recorded in RNK-1 (5.65 µm).

Sclerotial formation was initiated at 3 DAI for two isolates (NDK-1 and HGK-1) whereas, for the remaining isolates it was initiated at 4DAI. The distributions of sclerotia was observed in two different forms *i.e*., surface and touching the lid. Six isolates NDK-2, JDK-2, SGK-1, ARK-1, AMK-1, BAK-2 were fallen under surface and touch the lid category whereas, the sclerotial distribution for the remaining isolates were on the surface of the petriplates. Additionally, the fifteen isolates displayed considerable variation in the arrangement patterns of the sclerotial bodies. Majority of the isolates exhibited sub-central ring pattern (NDK-1, JDK-1, HGK-1, BAK-1) whereas other isolates exhibited central, peripheral ring, irregular, scattered and a combination of central and scattered pattern of sclerotial arrangement.

The number of sclerotia produced among the isolates varied from 54.67 in VZK-2 to 191.67 in SGK-1, the sclerotial weight ranged from 28.3 mg in BLK-2 to 85.2 mg in MDK-1. Concerning the sclerotial texture, majority of the isolates (VZK-1, NDK-2, JDK-1, JDK-2, SGK-1, ARK-1, RNK-1, KRK-1, HGK-1, BAK-1) formed rough textured in contrast to the remaining five isolates (VZK-2, NDK-1, MDK-1, AMK-1, BAK-2) which has formed smooth textured sclerotial bodies. These morpho-cultural observations highlight the significant variation present across the isolates.

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